

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1-30. (Canceled)

31. (Currently Amended) A method for detecting the presence of anti-MHC antibodies in a sample, the method comprising the steps of:

providing a substrate;

obtaining a pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes that have been purified substantially away from other proteins, each complex present in the pool comprising the same truncated, individual MHC heavy chain molecule, the pool obtained by the steps of:

isolating mRNA from a source, wherein the mRNA encodes ~~at least one~~ an MHC heavy chain allele;

reverse transcribing the mRNA to obtain cDNA;

identifying an individual MHC heavy chain allele in the cDNA;

PCR amplifying the individual MHC heavy chain allele in a locus-specific manner to produce a PCR product having the coding regions encoding cytoplasmic and transmembrane domains of the individual MHC heavy chain allele removed such that the PCR product encodes a truncated, soluble form of the individual MHC heavy chain molecule;

cloning the PCR product into a mammalian expression vector, thereby forming a construct that encodes the individual soluble MHC heavy chain molecule;

transfecting a mammalian cell line with the construct to provide a mammalian cell line expressing a construct that encodes a recombinant, individual soluble MHC heavy chain molecule, wherein the mammalian cell line is

able to naturally process proteins into peptide ligands for loading into antigen binding grooves of MHC molecules, and wherein the mammalian cell line expresses multiple surface-bound native Class I endogenous MHC molecules;

culturing the mammalian cell line under conditions which allow for expression of the recombinant individual soluble MHC heavy chain molecule from the construct, such conditions also allowing for endogenous loading of a peptide ligand into the antigen binding groove of each individual soluble MHC heavy chain molecule in the presence of beta-2-microglobulin to form the individual soluble MHC trimolecular complexes prior to secretion of the individual soluble MHC trimolecular complexes from the cell, wherein each trimolecular complex comprises a recombinant, soluble MHC heavy chain allele, beta-2-microglobulin and endogenously loaded peptide and wherein each trimolecular complex of the pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes has the same recombinant, soluble MHC heavy chain allele; and

purifying the individual, soluble MHC trimolecular complexes substantially away from other proteins, wherein the individual soluble MHC trimolecular complexes maintain the physical, functional and antigenic integrity of the native MHC trimolecular complex;

linking ~~at least one~~ a soluble MHC trimolecular complex to a substrate, wherein the ~~at least one~~ soluble MHC trimolecular complex is directly or indirectly linked to the substrate, and wherein the ~~at least one~~ soluble MHC trimolecular complex linked to the substrate retains the physical, functional and antigenic integrity of the native MHC trimolecular complex;

providing a sample;

reacting the sample with the substrate having the ~~at least one~~ MHC trimolecular complex linked thereto;

washing the substrate to remove unbound portions of the sample;
reacting the substrate having the ~~at least one~~ MHC trimolecular complex linked thereto
with means for detecting anti-MHC antibodies; and
determining that anti-MHC antibodies specific for the individual MHC molecule are
present in the sample if the means for detecting anti-MHC antibodies is positive.

32. (Previously Presented) The method of claim 31 wherein, in the step of providing a substrate, the substrate is a solid support.

33. (Previously Presented) The method of claim 32 wherein the solid support is selected from the group consisting of a well, a bead, a membrane, an ELISA plate, and a matrix.

34. (Previously Presented) The method of claim 33 wherein the bead is selected from the group consisting of a flow cytometry bead, and a magnetic bead, and wherein the membrane is selected from the group consisting of a nitrocellulose membrane, a PVDF membrane, a nylon membrane, and acetate derivative.

35. (Currently Amended) The method of claim 31 wherein, in the step of linking ~~at least one~~ a soluble MHC trimolecular complex to a substrate, the ~~at least one~~ soluble MHC trimolecular complex is indirectly attached to the substrate via an anchoring moiety.

36. (Previously Presented) The method of claim 35 wherein the anchoring moiety comprises an antibody to the functionally active, individual soluble MHC trimolecular complex.

37. (Previously Presented) The method of claim 36 wherein the antibody is selected from the group consisting of W6/32, anti-beta 2m, pan-Class I or allele-specific antibodies and combinations thereof.

38. (Currently Withdrawn - Previously Presented) The method of claim 35 wherein the anchoring moiety comprises a tail or tag attached to the functionally active, individual soluble MHC trimolecular complex, and the substrate is further defined as comprising an affinity reagent to which the tail or tag binds.

39. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a histidine tag, and the affinity reagent is selected from the group consisting of nickel, copper and combinations thereof.

40. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a biotinylation signal peptide, and the affinity reagent is avidin or streptavidin.

41. (Currently Withdrawn) The method of claim 38 wherein the tail or tag is a VLDLr or FLAG tail, and the affinity reagent is an antibody that recognizes the VLDLr or FLAG tail.

42. (Previously Presented) The method of claim 31 wherein, in the step of providing a pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes, the pool of functionally active, recombinantly produced, truncated individual soluble MHC trimolecular complexes are Class I or Class II MHC trimolecular complexes.

43-44. (Canceled)

45. (Previously Presented) The method of claim 31 wherein, in the step of isolating mRNA from a source, the source is selected from the group consisting of mammalian DNA and an immortalized cell line.

46. (Previously Presented) The method of claim 31 wherein, in the step of cloning the PCR product into a mammalian expression vector, the mammalian expression vector contains a promoter that facilitates increased expression of the truncated PCR product.

47. (Canceled)

48. (Previously Presented) The method of claim 31 wherein, in the step of PCR amplifying the individual MHC heavy chain allele, a primer utilized in the PCR amplification includes a sequence encoding a tail such that the soluble MHC heavy chain molecule encoded by the truncated PCR product contains a tail attached thereto that facilitates in purification of the soluble MHC trimolecular complexes produced there from or facilitates in direct binding of the soluble MHC trimolecular complexes to the substrate.

49. (Previously Presented) The method of claim 31 wherein, in the step of PCR amplifying the individual MHC heavy chain allele, a 3' primer utilized in the PCR amplification includes a stop codon incorporated therein.

50. (Previously Presented) The method of claim 31 wherein, in the step of purifying the individual, soluble MHC trimolecular complexes substantially away from other proteins, the functionally active, individual soluble MHC trimolecular complexes are purified by affinity chromatography and fractionation.

51. (Previously Presented) The method of claim 50 wherein the affinity chromatography utilizes a reagent selected from the group consisting of W6/32 antibodies, anti-b2m antibodies, pan-Class I antibodies or allele-specific antibodies, and combinations thereof.

52-59. (Canceled)

60. (Previously Presented) The method of claim 31 wherein, in the step of providing a sample, the sample is selected from the group consisting of serum, tissue, blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid, organ or tissue culture derived fluids, fluids extracted from physiological tissues, and combinations thereof.

61. (Currently Amended) The method of claim 31 wherein, in the step of reacting the substrate having the ~~at least one~~ MHC trimolecular complex linked thereto with means for detecting anti-MHC antibodies, the means for detecting anti-MHC antibodies is a labeled anti-human antibody recognizing human IgG, IgM or IgA antibodies.

62-92. (Canceled)